Relative contributions of α-, β-, and ω-oxidative pathways to in vitro fatty acid oxidation in rat liver

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ABSTRACT A method is presented for estimating the relative contribution of α-, β-, and ω-oxidation to the metabolism of fatty acids. The distribution of ^{14}C among the carbon atoms of liver glycogen-glucose is determined after liver slices have been incubated with fatty acids labeled at single points in the chain. When these results are compared with similar data obtained after an incubation with acetate-1-^{14}C and acetate-2-^{14}C, conclusions can be drawn about the contributions of α-, β-, and ω-oxidation in palmitate, oleate, and stearate, and of oxidative cleavage of the double bond in oleate. β-Oxidation is overwhelmingly predominant under the conditions used.

KEY WORDS fatty acid oxidation · α-, β-, ω-pathways · unsaturated · oxidative cleavage · liver · rat · in vitro · palmitate-1-^{14}C · palmitate-16-^{14}C · stearate-18-^{14}C · oleate-10-^{14}C

THE LONG-STANDING HYPOTHESIS (1) that β-oxidation is the major pathway for fatty acid catabolism has been amply confirmed, and the enzymes and intermediates involved in this process are well established (2, 3). ω-Oxidation and α-oxidation are also known to occur and these mechanisms have recently been elucidated (4–7). The relative contributions of these alternative degradative mechanisms in mammalian tissue have not yet been established (5).

We have developed a method for determining the relative contributions of the alternative oxidative pathways of fatty acid oxidation. The method depends upon a comparison of the fate of specific carbons of fatty acids with the fate of carbons 1 and 2 of acetate (8). Liver incorporates ^{14}C from acetate-1-^{14}C and -2-^{14}C into the glucose units of glycogen in a characteristic pattern: acetate-1-^{14}C contributes label to carbons 3 and 4 of the glucose; acetate-2-^{14}C, to carbons 1, 2, 5, 6, and to a lesser degree to carbons 3 and 4 (8). β-Oxidation of a fatty acid in which an even-numbered carbon atom is ^{14}C yields acetate-2-^{14}C, unless the acid first undergoes α-oxidation, when the product is acetate-1-^{14}C. β-Oxidation of an acid labeled at an odd-numbered position would yield acetate-1-^{14}C, as would ω-oxidation of a terminally labeled fatty acid, or the cleavage of a double bond whose carbons are labeled with ^{14}C followed by β-oxidation. Thus, preferential labeling of carbons 3 and 4 of glucose by oxidation of a fatty acid that had been labeled at an even-numbered carbon, or of an unsaturated fatty acid labeled at the even-numbered carbon that is at one end of the double bond, as compared with the labeling obtained with acetate-2-^{14}C, indicates oxidation of the acid by mechanisms other than β-oxidation.

METHODS AND MATERIALS

A paired flask technique was used in which slices of livers from two or three rats were distributed in flasks so that the same representation of slices was in each flask. Between 1.6 and 2.6 g of slices was added to each flask. The rats were male, of the Wistar strain, weighed 150–200 g, were fed a stock diet ad libitum, and had been fasted for 24 hr. In each flask there were 10 ml of a bicarbonate, high potassium medium (9) containing 50 μmoles of acetate, 100 μmoles of glucose, 20 μmoles of a fatty acid, and fatty acid-free albumin (Calbiochem, Los Angeles, Calif.). The molar ratio of fatty acid to al-

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bumin was 6:1 in experiments 1 through 3 and 3:1 in the remaining experiments. In each experiment, for each fatty acid studied, in one flask the fatty acid was labeled and in another flask the acetate was labeled, except that in experiments 3–5, where more than one fatty acid was studied, the unlabeled fatty acid was omitted from the flask containing labeled acetate. Similar distributions from acetate were found whether or not the unlabeled fatty acid was present. The contents of the flask were gassed with 95% O_2–5% CO_2 and then incubated with shaking at 37°C for 90 min.

Sodium acetate-1-^{14}C and -2-^{14}C, palmitic acid-16-^{14}C, and stearic acid-18-^{14}C were obtained from New England Nuclear Corp., Boston, Mass.; palmitic acid-1-^{14}C, from Volk Radiochemical Company, Burbank, Calif., and from New England Nuclear Corp.; oleic acid-10-^{14}C, from Commission d'Énergie Atomique, Département des Radioéléments, Gif-sur-Yvette, France. The materials were reported to be radiochromatographically pure. The fatty acids were rechromatographed on thin layers of Silica Gel G (E. Merck A. G., Darmstadt, Germany) in acetic acid–diethyl ether–petroleum ether (bp 38–50°C) 2:15:85 (10). The areas corresponding to spots given by unlabeled fatty acid in guide lanes were eluted with benzene, sealed under N_2, and stored at −20°C until used. The solvent was evaporated under N_2, and the fatty acids were neutralized with equivalent amounts of 0.023 N KOH and added to the albumin solution before the latter was combined with the rest of the medium. The pH of the final incubation medium, after gassing with 95% O_2–5% CO_2 and before addition of the tissue slices, was 7.3–7.4.

Incubations were terminated by the addition of 2 ml of 5 N H_2SO_4 and the tissues were then blotted and immersed in hot 33% KOH. Glycogen was precipitated by adding ethanol and then purified by several times dissolving it in water and reprecipitating it with ethanol. The glycogen was hydrolyzed in 1 N H_2SO_4 and the hydrolysate was deionized and concentrated. The glucose in the concentrate was degraded to yield each of its carbons as CO_2 by using *Leuconostoc mesenteroides* and then chemical methods. CO_2 was assayed for ^{14}C activity in a liquid scintillation counter (11).

In a sixth experiment, slices were incubated in paired flasks with acetate-1-^{14}C and -2-^{14}C in the presence of unlabeled palmitate, stearate, and oleate. The incorporation of ^{14}C into glycogen was determined by assaying glucosazones formed from the glycogens after their precipitation and hydrolysis (9).

RESULTS

The relative specific activities of the individual carbons are shown in Table 1. In all experiments, there was more activity in carbons 4, 5, and 6 of glucose than in carbons 1, 2, and 3. This finding is compatible with the occurrence in liver of exchange reactions via transaldolase (12), and with nonequilibration of triose phosphates (13).

TABLE 1 RELATIVE SPECIFIC ACTIVITIES IN GLUCOSE FROM GLYCOGEN OF LIVER SLICES, INCUBATED WITH THE VARIOUS ^{14}C-LABELED SUBSTRATES

Expt. No.	Substrate	Specific Activity* in Carbon No. 1	2	3	4	5	6
1	Acetate-2-^{14}C	52	53	14	21	96	100
2		52	52	15	14	96	100
3		64	64	19	31	80	100
5		60	60	17	26	105	100
Mean		57	57	16	23	94	100
3	Palmitate-16-^{14}C	59	69	24	24	82	100
5		55	54	20	21	97	100
Mean		57	62	22	23	90	100
2	Stearate-18-^{14}C	47	48	13	22	105	100
4		46	60	23	21	90	100
5		71	76	26	32	107	100
Mean		55	61	21	25	101	100
1	Oleate-10-^{14}C	49	52	14	22	102	100
3		56	57	17	26	92	100
5		64	62	17	19	93	100
Mean		56	57	16	22	96	100
2	Acetate-1-^{14}C	2	13	47	100	1	1
3		3	2	49	100	1	1
5		3	5	57	100	1	1
Mean		3	7	48	100	1	1
5	Palmitate-1-^{14}C	3	4	59	100	1	4

* Specific activities are relative to carbon 6 set to 100 for the first four substrates, and relative to carbon 4 set to 100 for the last two substrates.

In the incubations with acetate-2-^{14}C, palmitate-16-^{14}C, stearate-18-^{14}C, and oleate-10-^{14}C, there were similar degrees of activity in carbon 1 and 2 and in carbons 5 and 6, and more activity in each of these carbons than in either 3 or 4. ^{14}C from acetate-1-^{14}C and palmitate-1-^{14}C was incorporated essentially only into carbons 3 and 4.

About half as much ^{14}C from acetate-1-^{14}C as from acetate-2-^{14}C was incorporated into glycogen when these labeled substrates were incubated, under identical conditions, in the presence of the unlabeled fatty acids.

DISCUSSION

The degradation of glucose from glycogen formed from palmitate-1-^{14}C and acetate-1-^{14}C provides evidence that in our system a fatty acid which, whether metabolized via α-, β-, or ω-oxidation or via all three mechanisms, can yield only acetate-1-^{14}C, gives the distribution to be expected from that product (8).

From the remaining data of Table 1, the relative contributions of α-, β-, and ω-oxidation to the metabolism

of the fatty acids can be assessed. Compare, for instance, the data on stearate-18-^{14}C with those for acetate-2-^{14}C. Of the total quantity of ^{14}C from acetate-2-^{14}C incorporation into the glucose unit of glycogen, the fraction incorporated into carbons 3 and 4 is $(16 + 23)/(57 + 57 + 16 + 23 + 94 + 100) = 0.11$. If ^{14}C from stearate-18-^{14}C were incorporated into glucose solely via acetate-2-^{14}C, the same ratio would be expected for it. Actually, relatively more ^{14}C was incorporated into carbons 3 and 4 with stearate-18-^{14}C as substrate and the fractional excess is $[(21 + 25) - (16 + 23)]/(16 + 23) = 0.18$. Since the ^{14}C from acetate-1-^{14}C is incorporated almost exclusively into carbons 3 and 4 of the glucose unit of glycogen, 0.11 as much ^{14}C from acetate-1-^{14}C as -2-^{14}C would need to be incorporated to yield the same quantity of ^{14}C in carbons 3 and 4. Therefore, the number of ^{14}C atoms from acetate-1-^{14}C compared to those from acetate-2-^{14}C which would have to be incorporated into glycogen to yield the fractional excess of 0.18 is $0.18 \times 0.11 = 0.02$.

The above calculation does not indicate the actual quantity of acetate-1-^{14}C and -2-^{14}C formed from stearate-18-^{14}C, since for the same quantity of acetate-1-^{14}C as acetate-2-^{14}C utilized by liver slices, only half as much ^{14}C from acetate-1-^{14}C as from acetate-2-^{14}C is incorporated into glycogen. Therefore, the actual ratio is nearer 0.04. If ω- and β-oxidation are then assumed to be the only mechanisms that participated in the metabolism of stearate-18-^{14}C, twenty-five times as much acetate was formed from the terminal carbons of stearate via β-oxidation as via ω-oxidation.

When palmitate-16-^{14}C was the labeled fatty acid, the relative excess incorporation into carbons 3 and 4 was still lower than for stearate-18-^{14}C (Table 1). There was no excess incorporation of ^{14}C from oleate-10-^{14}C into carbons 3 and 4 of glucose, and the oxidation of this acid therefore seems to have been exclusively β-.

It must be remembered that before labeled carbons of stearate-18-^{14}C, palmitate-16-^{14}C, and oleate-10-^{14}C are liberated by cleavage a number of carbons can be oxidized and cleaved via β- and (or) α-oxidation. Although it seems improbable, two or any other even number of α-oxidations on the carbon chain preceding carbon 10 of oleic acid could occur with subsequent β-cleavage and still yield the results we observed. Similarly, if ω-oxidation were to occur, followed then by β-oxidation from the original carboxyl group of the fatty acid, the ω-oxidation would also go undetected. There are no data supporting such possibilities.

Our estimates are, at best, approximations because only a few degradations for each fatty acid have been performed. The degree of variation in the degradations precludes assignment of any significance to small differences in the sum of the incorporations into carbons 3 and 4 for the several fatty acids. The results do show that if α- and ω-oxidations contribute to the metabolism of palmitate, stearate, and oleate, and if oxidative cleavage of the double bond of oleate occurs in liver slices, these processes are very small to negligible in comparison to β-oxidation.

The approach used here to study the oxidative pathways for fatty acids in liver should be applicable to other tissues. Derivatives other than glucose from glycogen that show a difference between incorporation of ^{14}C from acetate-1-^{14}C and acetate-2-^{14}C should also be suitable as indicators.

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